

THE EFFECT OF LOW OXYGEN TENSION ON THE PULSA- TIONS OF THE ISOLATED HOLOTHURIAN CLOACA

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The sequence of events in the respiration of *Stichopus moebii* Semper has been adequately set forth by Crozier (1916). In laboratory aquaria the rhythmic activity of the cloaca is distinctly periodic. A series of several pulsations is followed by a pause during which water is expelled from the respiratory tree. Then another series of inspirations begins. The number of inspirations in a series was found by Crozier (1916) to range from five to eleven, the greatest number being found in the largest animal. Pearse (1908) pointed out that, if the respiratory pulsations of *Thyone briareus* are prevented for some time by repeated mechanical stimulations, the contractions which ensue when stimulation ceases are greatly augmented in amplitude.

Oxygen deficiency has often been associated with periodicity and augmentation of response in various tissues. Douglas and Haldane (1909) have described periodic breathing in man under low oxygen tensions, and Douglas (1910) found the same type of breathing at high altitudes. Magnus (1904) and Frey (1923) reported that a stoppage of the oxygen supply to beating smooth muscle results immediately in an increase in amplitude. The present paper deals with the phenomena which have been observed on decreasing the oxygen available to a rhythmically beating isolated strip of circular muscle from the cloaca of *Stichopus moebii* Semper. This holothurian is found in great numbers in the shore waters at the Bermuda Biological Station. During the summer of 1927 the author repeated some of the experiments on a ring preparation from the cloaca of *Cucumaria frondosa*, very abundant at the Mount Desert Island Biological Laboratory, Maine.

METHOD

Crozier (1916) has shown that the cloaca *in situ* in the isolated posterior end of *Stichopus* will maintain its pulsations for many hours. No reference to the use of an isolated strip of this organ could be

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found in the literature. The present work was carried out with an opened ring of the circular muscle of the cloaca. A cloacal-end preparation was first made similar to that described by Crozier (1916). The cloaca was then excised by cutting the radial muscles with a scalpel and freeing the organ from the anal rim by a transverse cut. From the muscular tube thus obtained a strip was made, one to two centimeters broad, and from four to six centimeters long. This strip was suspended vertically in a vessel of sea water by means of an L-shaped glass rod and a counterbalanced aluminum lever. A 250 cc. graduated cylinder cut off to hold about 125 cc. was found convenient as a vessel to hold measured amounts of sea water, or through which sea water could be made to flow continuously. The temperature of the water was recorded and found to vary little during an experiment, or from day to day. Therefore no special precautions for maintaining constant temperature were necessary.

RESULTS

Records were taken from strips of *Stichopus* cloaca beating under the following conditions: (1) in a continuous flow of sea water, (2) in a limited amount of sea water, (3) in boiled sea water with added carbon dioxide, (4) in boiled sea water of various degrees of aeration, and (5) in normal sea water with potassium cyanide added.

Continuous Flow of Sea Water.—When sea water was made to flow continuously through the vessel at the rate of about 100 cc. a minute, the strip beating therein gave a tracing which was exceedingly uniform over a period of several hours, as may be seen in Fig. 1. Both amplitude and tone increased during the first hour. This condition was maintained for an hour or more. Then the tone began to fall very gradually while the amplitude remained about the same. After five to seven hours from the beginning, the amplitude began to decrease slightly. The rhythm was exceedingly regular and no indications of periodicity appeared. The rate of beat decreased slowly from the start, in one case almost 50 per cent after seven hours and forty-one minutes; but the preparation was still vigorous and regular.

Limited Amount of Sea Water.—When a strip was allowed to beat in a limited amount of sea water, that is in 100 cc. without change, the amplitude began to increase in about three hours and distinct periodicity developed as seen in Fig. 2. The increase in amplitude continued for an hour or more, becoming 230 per cent in one case. The tone was maintained until the increase in amplitude occurred, when it gradually fell; but the increase in amplitude was not entirely due to a decrease in tone since the contractions of the strip raised the lever a greater

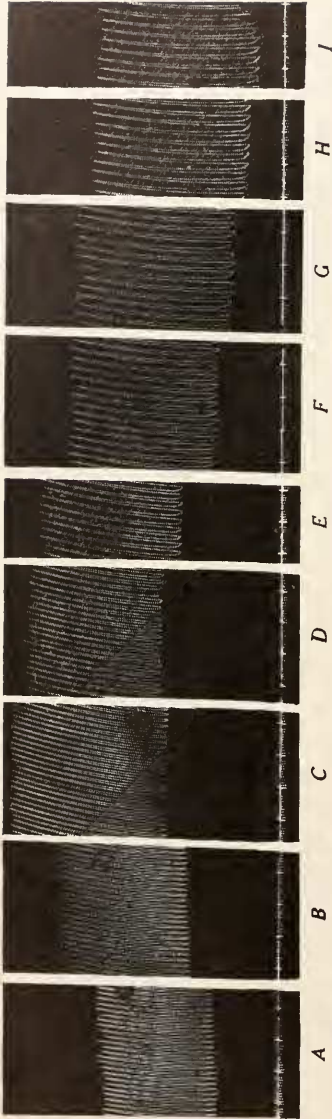


FIG. 1. Experiment 47. An isolated cloacal strip from *Stichopus macleodii* beating in sea water flowing continuously through the vessel. In this and subsequent tracings the large line markings on the base line mark time in minutes. Time after immersion of strip: *A*, 12 minutes; *B*, 45 minutes; *C*, 81 minutes; *D*, 139 minutes; *E*, 210 minutes; *F*, 300 minutes; *G*, 345 minutes; *H*, 390 minutes; *I*, 435 minutes. Duration of experiment 7 hours and 45 minutes.

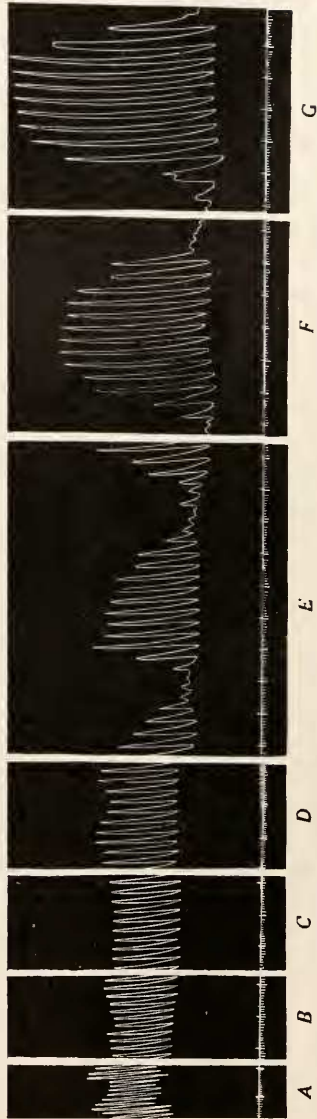


FIG. 2. Experiment 44. Cloacal strip beating in 100 cc. of sea water. Time after immersion of strip: *A*, 1 minute; *B*, 28 minutes; *C*, 60 minutes; *D*, 121 minutes; *E*, 161 minutes; *F*, 185 minutes; *G*, 222 minutes. Duration of experiment 3 hours and 48 minutes.

distance above the base line than in the beginning. Finally both amplitude and tone fell markedly. The rate of beat decreased constantly from five or six at the beginning to two or three per minute during the periods of beating. The length of the periods of inhibition of beat gradually increased to three or four minutes.

Boiled Sea Water.—Sea water which had been boiled in a narrow-necked flask and cooled to laboratory sea water temperature (28° C. or 29° C.) was used. When the muscle strip was immersed in 100 cc. of this water, the first two or three beats usually increased in amplitude, but both tone and amplitude almost immediately fell and the strip ceased beating in from three to five minutes as shown in Fig. 3, *A*. If the

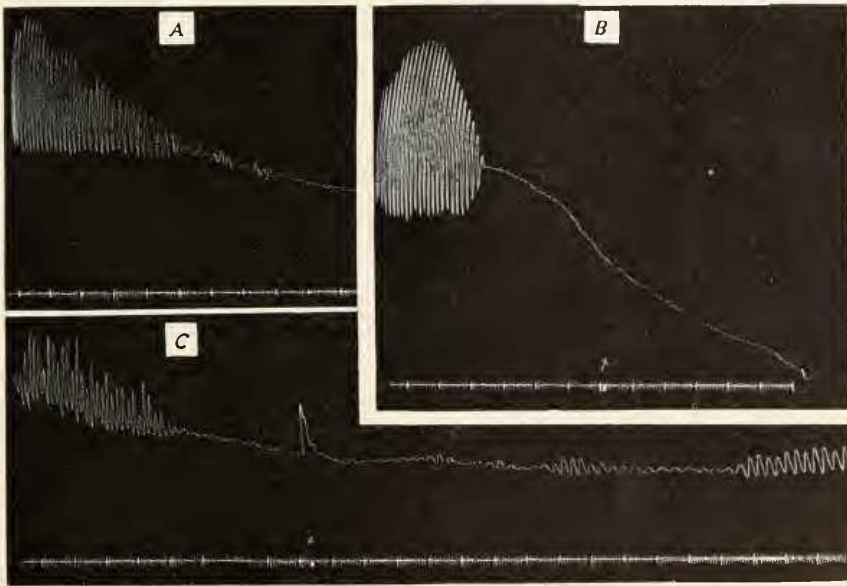


FIG. 3. *A*. Cloacal strip in 100 cc. of boiled sea water, pH 8.4. *B*. Cloacal strip in 100 cc. of boiled sea water treated with carbon dioxide, pH 5.8. Aeration at *X*. *C*. Cloacal strip in 100 cc. of boiled sea water, pH 8.3. Aeration at *X*. pH 8.2 immediately after aeration. pH 8.2 after 102 minutes.

water was aerated within three minutes by sucking it into a hypodermic syringe and squirting it back forcibly, a partial recovery occurred, which showed periodicity at first but later an uninterrupted rhythm (see Fig. 3, *C*). Several attempts to bring about recovery after waiting a longer period failed. The pH of the boiled sea water (indicator method) was sometimes as high as 8.8 as compared with 8.1 to 8.3, the pH for unboiled sea water in this region.

Boiled Sea Water with Added Carbon Dioxide.—Inasmuch as boiling removed the carbon dioxide as well as the oxygen, the former was replaced by means of a carbon dioxide generator. This resulted in boiled sea water ranging from pH 5.8 to 7.7. At the latter value the strip ceased to beat in three and one-half minutes and at the former value cessation occurred in three minutes. Aeration of the water after a three minute period of cessation failed to induce recovery (see Fig. 3, B). It seems therefore that neither the lack of carbon dioxide in the boiled sea water nor the increased alkalinity was the cause of the cessation of the pulsations.

A moderate excess of carbon dioxide was produced by treating 125 cc. of unboiled sea water with carbon dioxide until the pH was 7.0. This procedure was brief and probably did not remove much oxygen. In experiment 75 (Fig. 4) the amplitude began to decrease slowly after an hour, the rate decreasing gradually from the beginning. Neither augmentation of amplitude nor periodicity had appeared when the experiment was stopped after two hours and fifty-six minutes. When, however, an excess of carbon dioxide was produced by adding a few drops of N/10 HCl to a preparation beating in 100 cc. of unboiled sea water, there was an immediate rise in tone and increase in amplitude which soon gave way to a fall of tone and amplitude and finally to cessation of beat. It is therefore not probable that an accumulation of carbon dioxide in the immersion fluid as a result of tissue activity in a limited volume of water is the cause of the appearance of periodicity although it might be called upon to account for the increase in amplitude.

Boiled Sea Water of Various Degrees of Aeration.—When a decreased oxygen content of the sea water was produced, either by mixing boiled sea water with unboiled sea water or by partial aeration of boiled sea water, the augmentation and periodicity appeared much sooner than when a limited volume of unaltered sea water was used, the onset varying from a few minutes to two hours, according to the degree of oxygen lack. In one case the boiled sea water had been stored for several hours in a narrow-necked flask with only a few square centimeters of water surface exposed to the air. A strip beating in 100 cc. of this water became periodic at once and each successive period showed an increase in amplitude which finally amounted to about 200 per cent. The tone and the rate of beat, however, fell rapidly.

In another experiment in which 100 cc. of boiled sea water had been partially aerated, wave-like variations in amplitude appeared 13 minutes after immersion, and gradually developed into periodicity 53 minutes after the start. The amplitude increased from 10 mm. to 24



FIG. 4. Experiment 75. Cloacal strip in 125 cc. of sea water treated with carbon dioxide, pH 7.0. *A*, beginning at 10:00; *B*, 10:30; *C*, 11:00; *D*, 11:30; *E*, 12:00; *F*, 12:52. At the end, 12:56, pH 7.0.



FIG. 5. Experiment 62. Cloacal strip in 100 cc. of boiled and partially aerated sea water. Before boiling, pH 8.3. After boiling and partial aeration, pH 8.4. Time after immersion: *A*, 5 minutes; *B*, 32 minutes; *C*, 97 minutes; *D*, 117 minutes; *E*, 181 minutes. pH 8.4 after 166 minutes.

mm. and was still high when the experiment was stopped at the end of 93 minutes. The tone fell rapidly during the first five minutes and then more slowly during the next ten minutes after which it was unchanged. The rate decreased about fifty per cent during the first half hour and then remained constant.

In experiment 62 (Fig. 5) the boiled sea water (100 cc.) was partially aerated. Before boiling the pH was 8.3, but after boiling and partial aeration it was 8.4. The amplitude of a strip beating in this water decreased at first with a tendency to form waves. Then for a period of 80 minutes the amplitude remained constant, but at the end of this period the amplitude began to increase, becoming 65 per cent greater than that during the previous period of uniform amplitude. Periodicity appeared in about two hours from the beginning of the experiment, the number of beats in each period ranging from ten to sixteen, while the period of interruption varied from one minute and a quarter to three minutes. The pH was still 8.4 about forty-five minutes after periodicity and augmentation were well developed. Apparently these phenomena were not due to increased acidity of the surrounding medium, nor was the cessation of beat in the cases of extreme oxygen lack due to an increase in the concentration of the salts resulting from boiling.

When 75 cc. of boiled sea water were mixed with 25 cc. of unboiled water, the pH of the mixture was 8.8. The first few contractions increased in amplitude about 20 per cent, lasting for about three minutes. Then a fall occurred, and the amplitude remained uniform in height until waves in amplitude appeared in one hour indicating the onset of periodicity, which became well marked about half an hour later. At this time the pH was still 8.8.

Effect of Potassium Cyanide.—When ten drops (about 0.5 cc.) of *M/10* potassium cyanide were added to 100 cc. of sea water in which a strip had been beating for a few minutes, the results were similar to those obtained with partial aeration. An increase in amplitude occurred within two minutes which varied in different cases from 18 to 400 per cent. The tone increased at about the same time. Periodicity occurred within fifteen minutes. In one case it began in three minutes, and the rate of beat was increased about one beat per minute for a brief period after the addition of potassium cyanide. Finally the tone and amplitude fell and the strip ceased to beat (see Fig. 6).

An examination of the results presented above suggests that lack of oxygen is responsible for the appearance of the two chief phenomena noted. Since augmentation and periodicity did not occur with a continuous flow of water but did occur in three hours when the amount

of water was limited to 100 cc., one might expect that one or more of several factors were responsible, such as, an increase in carbon dioxide, an increase in unoxidized acids, a depletion of essential ions, or a depletion of oxygen. However, when the carbon dioxide content of sea water was increased at the beginning, the phenomena did not

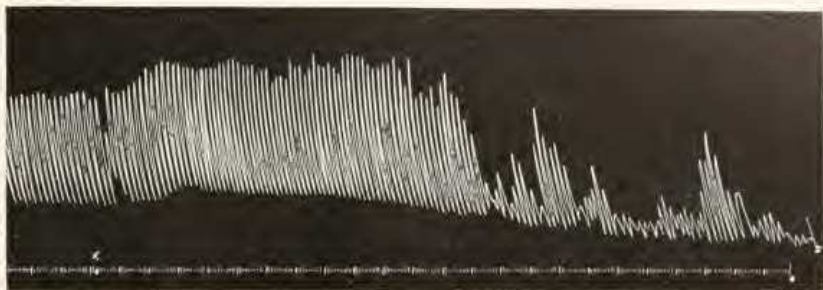


FIG. 6. Experiment 82. Cloacal strip in 100 cc. of sea water. Time of immersion 4:28. At X, 4:35, 20 drops of $M/10$ potassium cyanide added.

appear, although a temporary increase in amplitude and tone could be produced upon addition of hydrochloric acid. Moreover, in the experiments in which the phenomena did appear, the pH of the sea water was either unchanged or decreased very slightly. Since both augmentation and periodicity were made to appear much sooner when the water was partly depleted of oxygen at the beginning, or when potassium cyanide was added, the inference is that oxygen lack was either directly or indirectly responsible.

When a ring preparation made from the cloaca of *Cucumaria frondosa* was allowed to beat in a limited volume of sea water, namely, 25 or 30 cc., periodicity appeared in 50 minutes on the average in eight out of ten preparations. Two showed no periods. Augmentation of amplitude occurred in five cases. When boiled sea water was used the periodicity appeared in 25 minutes on the average in 14 out of 15 preparations. One showed no periods. Augmentation of amplitude occurred in 13 preparations. These results, especially when considered in the light of the results on *Stichopus*, indicate that lack of oxygen is a factor tending toward an early development of periodicity and augmentation.

DISCUSSION

Periodicity is a part of the normal respiratory sequence of a holothurian, the rhythmical contractions of the cloaca being inhibited while the body muscles squeeze out the sea water from the respiratory tree

through the relaxed anal valve. Crozier (1916), however, found no evidence of periodicity in the cloacal-end preparation of *Stichopus* and came to the conclusion that the stimulus for spouting has its origin outside the cloaca. It should be noted that he used larger volumes of water than were used in the work reported in this paper. Apparently no oxygen deficiency existed in his preparations, in which the cloacal pumping probably produced a sufficient movement of water to keep it aerated beyond the needs of the preparation. Since the isolated cloacal strip will exhibit regular periods of inhibition, the inference is that a part of the normal mechanism for spouting lies within the cloacal muscle. Since periodicity is lacking with sufficient aeration and appears quickly under conditions of oxygen deficiency, one is inclined to believe that low oxygen tension is a factor in determining the normal respiratory sequence in the holothurian.

Periodicity is commonly observed in the respiratory activity of vertebrates, as for example in the breathing of hibernating animals, in Cheynes-Stokes respiration, and in respiration at high altitudes. The causes of this phenomenon are usually associated with the chemical conditions in the respiratory center. Most authors have offered explanations which concern directly or indirectly the hydrogen ion concentration of the blood or fluid surrounding the cells. Gesell (1925), however, has called attention to the hydrogen ion concentration within the cells of the former, pointing out that when oxygen is present carbon dioxide is formed, but if oxygen is lacking lactic acid results. In either case the activity of the center increases as the acidity rises. Assuming a critical level, one needs further to call upon a mechanism for altering either the level or the acidity to account for periodic inhibition.

The augmentation of amplitude observed with a decrease in the available oxygen is in accord with the work of Magnus (1904) and of Frey (1923), who worked on vertebrate smooth muscle. Gross and Clark (1923), in an investigation on the influence of the oxygen supply on the response of the isolated intestine to drugs, stated that cutting off the oxygen resulted in a decrease in amplitude and tone. They did not comment on the immediate brief increase in amplitude and tone shown in their published graphs. The literature offers many additional observations which indicate that a certain degree of oxygen lack results in increased activity of tissue. Kaya and Starling (1909) found that lowering the oxygen tension resulted in excitation in the whole nervous system. Sherrington (1910) found that a certain degree of asphyxia favored the elicitation of the scratch reflex, and suggested that the hyperexcitability of the reflex was due to oxygen

lack. Mathison (1911) showed by the use of hydrogen, nitrogen, and carbon monoxide that the initial effect of oxygen lack on the medullary centers is clearly stimulating. Gasser and Lovenhart (1914) found by the use of carbon monoxide and sodium cyanide that decreased oxidation stimulated the medullary centers at first but later depressed them. Kellaway (1919) demonstrated that lack of oxygen may lead to stimulation of the adrenal glands, and Lutz and Schneider (1919) have observed a dilatation of the pupil in men during a period of breathing nitrogen. They also presented evidence to show that the cardiac and the respiratory medullary centers in man respond very quickly to changes in the partial pressure of oxygen. A decrease in oxygen tension increased the activity of these centers, while an increase in oxygen tension decreased their activity. Glazer (1929) found that intravenous injection of sodium cyanide in a dog increases the reflex response of the anterior tibial muscle, and Winkler (1929) obtained a similar effect with low alveolar oxygen tension.

In the muscle-and-nerve-net preparation reported in the present paper, it appears that the carbon dioxide content and the acidity of the surrounding fluid are not primary factors in controlling its activity. This conclusion is supported by the work of Hogben (1925) who found that, on adding acid to the perfused heart of *Maia* and of *Homarus*, the pH could be lowered from 7.0 to 5.6 without producing a change in the mechanical phenomena. Reduction beyond this point produced an immediate effect on the character of the rhythm. Nor could any alteration be noticed in the beating of the smooth muscle of *Helix* and of *Aplysia* on changing the pH from 7.0 to 6.0. In fact it is possible that the pH outside of the cell may vary markedly without greatly altering that inside of the cell. The oxygen tension appears to have some influence on tissue acidity. Frey (1923) presented evidence which shows that without oxygen the tissue rather than the surrounding fluid first changes its hydrogen ion concentration, and if this approaches the optimal value, an increased ability to respond ensues. The anaerobic production of acid in cellular activity and the rôle of oxygen in the recovery process suggest that oxygen lack is acting indirectly when cellular activity is first increased and is subsequently depressed.

SUMMARY

1. An isolated muscle strip from the cloaca of *Stichopus moebii* Semper and a ring of muscle from the cloaca of *Cucumaria frondosa* were used in sea water as rhythmically beating preparations.

2. In a continuous flow of sea water the contractions (*Stichopus*) were nearly uniform in rate and amplitude over a period of several hours, but a gradual decrease in both finally occurred.

3. In a limited volume of sea water (100 cc.) the amplitude (*Stichopus*) began to increase after three hours and a distinct periodicity of the regular rhythm developed. In the case of the cloacal ring of *Cucumaria* beating in 25 or 30 cc. of sea water, periodicity appeared in 50 minutes on the average.

4. In boiled sea water the strip (*Stichopus*) ceased beating in from three to five minutes, but partial recovery took place if the water was aerated within three minutes. If the carbon dioxide was replaced in the boiled sea water, cessation of beat occurred as before. In 25 or 30 cc. of boiled sea water the ring of cloacal muscle from *Cucumaria* developed periodicity in 25 minutes on the average.

5. A moderate excess of carbon dioxide in sea water (pH 7.0) did not bring on augmentation nor produce periodicity.

6. In boiled sea water of various degrees of aeration the augmentation of amplitude and the periodicity appeared sooner than in unboiled sea water. When little oxygen was present both phenomena appeared almost immediately, while the pH of the surrounding fluid was unchanged.

7. When potassium cyanide was added to the sea water an increase in amplitude and tone occurred and periodicity appeared.

8. Evidence from the literature is cited supporting the view that decreased oxygen tension results at first in increased activity of muscular and nervous tissues. This view is further supported by the evidence presented in this paper.²

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